

**[0077]** The term “attach” or “attached” as used herein, refers to connecting or uniting by a bond, link, force or tie in order to keep two or more components together, which encompasses either direct or indirect attachment such that for example where a first molecule is directly bound to a second molecule or material, and the embodiments wherein one or more intermediate molecules are disposed between the first molecule and the second molecule or material.

**[0078]** The term “substrate” as used herein indicates an underlying support or substratum. Exemplary substrates include solid substrates, such as glass plates, microtiter well plates, magnetic beads, silicon wafers and additional substrates identifiable by a skilled person upon reading of the present disclosure.

**[0079]** In some embodiments, the capture agents used in the arrays, devices, methods and systems herein disclosed can be either directly deposited onto substrate to form an array or immobilized by linker molecules that are pre-deposited onto substrate and capable to specific binding to capture agent for form an array. Since they are functional to the attachment of capture agents to a substrate, linker molecules can be considered as capture agent components.

**[0080]** In the arrays, substrates, devices, methods and systems herein disclosed, wherein multiple capture agents are used, each capture agent can be bindingly distinguishable and/or positionally distinguishable from another.

**[0081]** The wording “bindingly distinguishable” as used herein with reference to molecules, indicates molecules that are distinguishable based on their ability to specifically bind to, and are thereby defined as complementary to a specific molecule. Accordingly, a first molecule is bindingly distinguishable from a second molecule if the first molecule specifically binds and is thereby defined as complementary to a third molecule and the second molecule specifically binds and is thereby defined as complementary to a fourth molecule, with the fourth molecule distinct from the third molecule.

**[0082]** The wording “positionally distinguishable” as used herein refers to with reference to molecules, indicates molecules that are distinguishable based on the point or area occupied by the molecules. Accordingly, positionally distinguishable capture agents are substrate polynucleotide that occupy different points or areas on the assaying channel and are thereby positionally distinguishable.

**[0083]** In arrays herein disclosed, each capture agent of the plurality of capture agents is capable of specifically binding each target of the plurality of targets to form a capture agent target binding complex, and the plurality of capture agents arranged on the array so that capture agent target binding complexes are detectable along substantially parallel lines forming a barcoded pattern.

**[0084]** In other embodiments, substrates systems and methods are herein disclosed wherein the substrate is configured to allow attachment of targets (herein also reverse barcode or inversed-phase barcode), and in particular detectable targets, along substantially parallel lines forming a barcoded pattern. An exemplary illustration of reverse barcode is illustrated in FIG. 1, wherein a barcoded pattern including a number of bars corresponding to immobilized serum molecules from various patients and microfluidic channels for providing various drugs to be contacted with the serum of the patients for a bio-assay, are shown.

**[0085]** In some embodiments, detection of the attached target and/or capture agent target complex is performed by pro-

viding a labeled molecule, which includes any molecule that can specifically bind a capture agent target complex to be detected (e.g. an antibody, aptamers, peptides etc) and a label that provides a labeling signal, the label compound attached to the molecule. The labeled molecule is contacted with the attached target and/or capture agent target complex and the labeling signal from the label compound bound to attached target and/or the capture agent-target complex on the substrate can then be detected, according to procedure identifiable by a skilled upon reading of the present disclosure and, in particular, of the Examples section.

**[0086]** In particular, the signal readout that is used in the arrays, devices, methods and systems herein disclosed can be realized using labels such as probes that transduce the capture event of target molecule into optical, electrical or magnetic signal. Exemplary probes include, but not limited to, fluorescent dyes, gold nanoparticles, silver nanoparticles, semiconductor nanoparticles (e.g. CdSe, ZnSe and/or their core-shell nanoparticles), and iron oxide nanoparticles.

**[0087]** The terms “label” and “labeled molecule” as used herein as a component of a complex or molecule refer to a molecule capable of detection, including but not limited to radioactive isotopes, fluorophores, chemoluminescent dyes, chromophores, enzymes, enzymes substrates, enzyme cofactors, enzyme inhibitors, dyes, metal ions, nanoparticles, metal sols, ligands (such as biotin, avidin, streptavidin or haptens) and the like. The term “fluorophore” refers to a substance or a portion thereof which is capable of exhibiting fluorescence in a detectable image. As a consequence the wording and “labeling signal” as used herein indicates the signal emitted from the label that allows detection of the label, including but not limited to radioactivity, fluorescence, chemoluminescence, production of a compound in outcome of an enzymatic reaction and the likes.

**[0088]** In embodiments wherein one or more targets and/or a plurality of targets is detected described below in more details, the labeled molecule can be formed of a plurality of labeled molecules. Each labeled molecules comprises a molecule that specifically binds one target of the one or more targets/plurality of targets and a label compound attached to the molecule, the label compound providing a labeling signal, each labeled molecule detectably distinguishable from another.

**[0089]** The wording “detectably distinguishable” as used herein with reference to labeled molecule indicates molecules that are distinguishable on the basis of the labeling signal provided by the label compound attached to the molecule. Exemplary label compounds that can be used to provide detectably distinguishable labeled molecules, include but are not limited to radioactive isotopes, fluorophores, chemoluminescent dyes, chromophores, enzymes, enzymes substrates, enzyme cofactors, enzyme inhibitors, dyes, metal ions, nanoparticles, metal sols, ligands (such as biotin, avidin, streptavidin or haptens) and additional compounds identifiable by a skilled person upon reading of the present disclosure.

**[0090]** In embodiments, wherein bindingly distinguishable capture agents are used different analytes can be detected by use of detectably distinguishable labeled molecules each specific to a separate analyte of interest.

**[0091]** In some embodiments, the detection method can be carried via fluorescent based readouts, in which the labeled antibody is labeled with fluorophore which includes but is not limited to small molecular dyes, protein chromophores and quantum dots. In other embodiments, on-chip detection can